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THE LIPOTROPIC ACTION OF METHIONINE

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In 1937 Tucker & Eckstein demonstrated that methionine exerts a lipotropic effect. This has been confirmed in our laboratory [Best & Ridout, 1938] and by Channon, Manifold & Platt [1938]. The latter workers have shown that methionine, under the conditions of their experiments, exerted very little effect upon the deposition of fat in the liver unless the basal diet was such that large amounts of fat were deposited in the livers of the control animals. We have been interested in the lipotropic effects of *d*- and *l*-methionine and in the failure of large doses of the racemic mixture to produce greater effects than small doses under certain experimental conditions.

Methods

White rats of the Wistar strain, av. wt. 200 g., were used. The liver fat was estimated by direct saponification and the results are expressed as total fatty acids plus unsaponifiable matter per 100 g. fresh tissue. Animals of the same sex were used throughout an experiment and the figures given in the tables are the average values from groups of fifteen animals. The constituents of the basal diet are listed in Table V. The diets were all supplemented by adequate amounts of crystalline vitamin B_1 and vitamins A and D in the form of a cod-liver oil concentrate. The substance to be tested was added to the basal diet and a corresponding decrease in the amount of sucrose was made. The experimental period was 21 days. Daily records of the food consumption were made and from these the actual intake of the supplement was calculated.

Results

The results of the first experiment are summarized in Table I. They demonstrate that under these experimental conditions the lipotropic

TABLE I

Diet	Av. daily food intake g.	Av. daily intake of supplement	Av. change in wt. %	Liver fat: g. per 100 g. rat (calc.)	Liver fat: %
Basal	9·2	—	−10	0·58	15·2
+0·125% *dl*-methionine	9·5	11·9 mg.	− 6	0·47	11·6
+0·25% ,,	9·7	24·2 ,,	− 5	0·42	10·0
+0·50% ,,	9·4	47·0 ,,	− 4	0·37	9·4
+1·00% ,,	9·7	97·0 ,,	− 9	0·44	10·5
+ 8·0% casein	9·5	0·76 g.	+ 3	0·62	14·2
+16·0% ,,	9·6	1·54 ,,	+ 9	0·42	9·6
+32·0% ,,	9·0	2·88 ,,	+ 3	0·25	6·2

effect of a diet containing 1% *dl*-methionine is not significantly greater than that of one with 0·125%.

Dietary casein which contains approximately 3·2% of methionine [Baernstein, 1932] has previously been shown to exert a definite lipotropic action. In this experiment 47 mg. of methionine daily exerted about the same effect as the diet containing 16% casein which provided approximately 49 mg. of this amino-acid. On the other hand, at lower or higher levels of intake of the two substances, the effects were by no means identical.

As mentioned above, Channon and his collaborators showed that the lipotropic effect of methionine was much greater when the basal diet produced a high level of liver fat in the control animals. The results in Table II show that the absence of additional effects with larger doses of methionine

TABLE II

Diet	Av. daily food intake g.	Av. daily intake of supplement	Av. change in wt. %	Liver fat: g. per 100 g. rat (calc.)	Liver fat: %
Basal	9·2	—	− 6	0·89	20·5
+0·50% *dl*-methionine	9·3	46·5 mg.	− 1	0·55	13·3
+1·00% ,,	9·4	94·0 ,,	− 6	0·64	15·5
+2·00% ,,	9·5	190·0 ,,	−11	0·68	16·5
+16% casein	9·0	1·44 g.	+ 2	0·30	8·5
+32% ,,	8·6	2·76 ,,	+ 4	0·23	7·0
+0·08% choline	9·9	7·9 mg.	− 6	0·30	8·3
+0·16% ,,	9·3	14·9 ,,	− 5	0·24	6·6

can still be demonstrated when the control value of liver fat is high. The diet containing 2% *dl*-methionine did not lower the liver fat as much as the one with 0·5%. In this experiment the diet containing 16% casein caused a greater decrease in liver fat than could be attributed to its methionine content. The choline equivalent of the dietary casein was about 5 mg. per g. casein. This value is the same as that previously determined.

TABLE III

Diet	Av. daily food intake g.	Av. daily intake of supplement mg.	Av. change in wt. %	Liver fat g. per 100 g. rat (calc.)	Liver fat %
Basal	8·0	—	−11	1·06	25·7
+0·06% *dl*-methionine	8·7	5·2	− 5	0·82	18·0
+0·50% ,,	8·3	41·5	− 3	0·57	13·4
+0·06% *d*-methionine	9·2	5·5	0	0·94	19·7
+0·50% ,,	8·6	43·0	− 4	0·44	10·9
+0·06% *l*-methionine	8·9	5·3	− 4	0·69	16·0
+0·08% choline	8·3	6·6	− 6	0·21	5·9
+0·02% ,,	8·1	1·6	−10	0·58	16·3
+0·01% ,,	8·9	0·9	−14	0·71	17·7
Basal	9·5	—	−10	0·77	18·3
+0·06% *dl*-methionine	9·3	5·6	− 8	0·56	14·3
+0·06% *d*-methionine	8·5	5·1	− 8	0·60	14·3
+0·06% *l*-methionine	9·5	5·7	− 8	0·57	14·3
Basal	8·8	—	− 6	0·73	18·6
+0·15% *dl*-methionine	9·3	13·9	− 4	0·73	16·7
+0·25% ,,	9·1	22·7	− 4	0·63	16·0
+0·15% *d*-methionine	8·8	13·2	− 4	0·60	15·6
+0·25% ,,	8·7	21·7	− 5	0·49	13·7
+0·15% *l*-methionine	8·7	13·0	− 5	0·57	14·7
+0·25% ,,	9·1	22·7	− 5	0·57	14·9

In the third experiment (Table III) the effects of *d*- and *l*-methionine are compared with that of the racemic mixture and with various doses of choline. The results indicate that both *d*- and *l*-methionine are active and that their effects are essentially similar to that of the mixture.

TABLE IV

Diet	Av. daily food intake g.	Av. daily intake of supplement	Av. change in wt. %	Liver fat g. per 100 g. rat (calc.)	Liver fat %
Basal	9·5	—	−6	0·73	18·6
(1) +30% casein	8·5	2·55 g.	+3	0·25	7·1
(2) +0·15% choline	8·7	13·0 mg.	−5	0·24	7·8
(3) +0·10% cystine	8·4	84·0 ,,	−5	0·71	16·8
+0·96% *dl*-methionine		80·7 ,,			

In the last experiment (Table IV) cystine, which has been shown to increase liver fat [Curtis & Newburgh, 1927; Beeston & Channon, 1936], and methionine were both added to the basal diet in amounts equivalent to those provided by a diet containing 30% casein [Tucker & Eckstein, 1937]. The effect of this diet was then compared with one containing 30% casein and also with one in which choline was added to the basal diet in an amount equal to the choline equivalent of the casein. The results in Table IV show that the mixture of cystine and methionine exerts an insignificant effect in comparison with the casein diet which provided the same amounts of these amino-acids. The composition of the basal diet and of the others referred to in Table IV is described in Table V.

TABLE V. Composition of diets listed in Table IV

	Basal %	(1) %	(2) %	(3) %
Meat powder*	5	5	5	5
Casein†	—	30	—	—
Cystine	—	—	—	0·10
dl-Methionine	—	—	—	0·96
Beef dripping	40	40	40	40
Sucrose	48	18	47·85	46·94
Agar	2	2	2	2
Salt mixture‡	5	5	5	5
Choline	—	—	0·15	—
Cod-liver oil concentrate	+	+	+	+
Vitamin B_1	+	+	+	+

* For preparation, see MacLean, D. L., Ridout, J. H. & Best, C. H. [1937].
† Fat-free and vitamin-free, obtained from British Drug Houses.
‡ See McCollum, E. V. & Simmonds, N. J. [1918].

Discussion

Our results confirm and extend those reported by Tucker & Eckstein [1937] and by Channon *et al.* [1938]. There does not appear to be a quantitative relationship between the amount of methionine ingested and the deposition of fat in the liver. The lipotropic activity of the diet containing 2% methionine was no greater than the one with 0·5%. However, if the amount of methionine is reduced to 0·06%, as in the experiments recorded in Table III, the falling off in lipotropic effect is very definite.

Channon, Loach, Loizides, Manifold & Soliman [1938] demonstrated that the lipotropic activity of protein supplements varied with the nature and amount of the basal protein. In the experiments reported in this paper, meat powder was used as the basal protein. It is possible that an increase in the dose of methionine might produce a further decrease in liver fat if a different protein were used in the basal diet.

Tucker & Eckstein [1937] at one time suggested that the lipotropic effect of a diet containing 30% casein as the only source of dietary protein was due to the opposing influences of its cystine and methionine contents. The results of their more recent experiments and of those we are now reporting do not support this interpretation. If 30% casein is added to a diet containing 5% meat powder as the basal protein, a definite decrease in liver fat occurs. If cystine and methionine are added to the basal diet in amounts corresponding to those supplied by the diet containing 30% casein, there is little or no decrease in liver fat. Furthermore, the amount of liver fat is decreased when the amount of dietary casein is increased, but when additional quantities of methionine are added to the basal diet the lipotropic activity does not increase beyond a certain limit. This limit is reached under our experimental conditions when there are still large amounts of fat in the liver. If the lipotropic action of certain proteins is due to their constituent amino-acids, the results of these experiments suggest that other amino-acids are involved in the lipotropic activity of dietary casein. Tucker & Eckstein [1938] did not obtain an increase in liver fat when cystine was added to a diet which contained 5% gliadin as the basal protein, and they suggest that other amino-acids may exert the same effects as cystine or methionine. Our findings are therefore in agreement with those of Channon *et al.* [1938] and of Tucker & Eckstein [1938] who have emphasized the probability that other factors in addition to cystine and methionine may be involved in the explanation of the lipotropic effect of protein. Some light may be thrown on the problem by the further developments of the investigations of Du Vigneaud, Chandler, Moyer & Keppel [1939] and of Channon *et al.* [1938]. The effect of homocystine and related substances on fat metabolism is being studied by both these groups.

Summary

1. Methionine exerts a definite lipotropic action (confirming Tucker & Eckstein).

2. The activities of *d*-methionine, *l*-methionine and the racemic mixture are of the same order under our experimental conditions.

3. In experiments in which relatively small doses of methionine have produced a significant fall in liver fat, increase in the dose has not caused a further decrease in spite of the fact that large amounts of fat were still present in the liver.

4. Our results support the conclusion of other workers in this field that factors other than cystine and methionine are involved in the explanation of the lipotropic effect of dietary protein.

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